**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Appl. No. | : | **10/007,459** |
| Applicants | : | **David L. Lewis et al.** |
| Filed | : | **11/07/2001** |
| Art Unit | : | **1635** |
| Examiner | : | **Gibbs, Terra C.** |
| Docket No. | : | **Mirus.030.04** |

For: **Inhibition of Gene Expression by Delivery of Small Interfering RNA to Post-Embryonic Animal Cells *In Vivo***

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 C.F.R. §1.132**

Dear Sir:

We, Vladimir Subbotin, So Wong, Julia Hegge, Jon A. Wolff, and James Hagstrom, hereby declare as follows:

1. Vladimir Subbotin has an MD/PhD from Novosibirsk Medical School and over 35 years experience in animal pathology.

   So Wong has a PhD in Pharmacology from East Carolina University and over 15 years experience in the drug delivery field.

   Julia Hegge has a Bachelor's degree in Biology and Medical Technology from Edgewood College and over 20 year experience in the Medical Technology field.

   Jon Wolff has an M.D. from Johns Hopkins University and over 17 years experience in the gene delivery field.

   Jim Hagstrom has a PhD in Molecular biology from the Mayo Graduate School of Medicine and has over 14 years experience in the gene delivery field.

2. We are familiar with the above captioned application and with the Zimmer et al. (Methods in Enzymology 1999) reference cited in the Office Action.

3. In addition to its extensive role in metabolism, the liver also functions as a filter to remove potentially harmful substances from the blood. To aid in these functions, the vascular epithelium of the liver is discontinuous, i.e. it is fenestrated. Fenestrae are pores in vessels which allow for rapid exchange of molecules between blood vessels and surrounding liver tissue. In general, liver endothelial fenestrae measure 150–175 nm in diameter and occur at a frequency of 9–13 per $\mu m^2$. Thus, the liver vasculature does not present a mechanical barrier for small particles. As a result, a variety of molecules and molecular aggregates accumulate in liver tissue regardless of volume and speed of injection. An increase in vascular permeability is not required for molecules and complexes which enter the circulation to accumulate in the liver, usually in Kupffer cells. Kupffer cells are macrophages that line the liver sinusoids, forming part of the reticuloendothelial system, and function principally in phagocytosis of potentially obstructive debris, old erythrocytes, cellular fragments and bacteria that may arrive in the portal blood. Similarly, because of the structure of the kidneys and bone marrow, it is possible for molecules and complexes which enter the circulation to accumulate to detectable levels in these tissues.

Zimmer et al. teach injection of oligonucleotide nanoparticles into the tail vein of mice. The Action states that the volume used by Zimmer et al. inherently causes increased permeability in the target tissue, as evidenced by localization of the injected oligonucleotide in the liver. Zimmer et al. teach an injection of 5 nmol/5 ml/kg. Since an average mouse weighs about 25 grams (0.025 kg) this volume equates to 0.125 ml (5 ml/kg × 0.025 kg = 0.125 ml; i.e. 125 µl) per mouse. Based on our experience and knowledge of mouse physiology as well as that of the gene therapy and drug delivery arts, tail vein injection of this volume is insufficient to result in an increase in vasculature permeability within the liver, kidneys, bone marrow or other tissues. The accumulation of oligonucleotide in the liver, kidney, and bone marrow observed by Zimmer et al. is the result of the structural and functional organization of these tissues and not an increase in permeability caused by the injection.

Increasing vessel permeability via elevated hydrostatic pressure, as taught in U.S. Application No. 10/007,459, increases the efficiency of delivery of molecules and complexes to liver, kidney and bone marrow, and enables delivery to tissues that do not have discontinuous or porous epithelia. By injecting a larger volume at a higher rate than was done in the prior art, the vessels within a target tissue are subjected to increased hydrostatic pressure. This pressure

results in the solution and its contents moving out of the vessel and ***into*** cells in the surrounding tissue. The procedure taught by Zimmer et al. will not result in pressure mediated delivery of molecules or complexes into parenchymal cells.

4. We submit with this Declaration and Response further experimental material (below) illustrating the effect of volume on increasing permeability in liver vessels following tail vein injection. The experiments were performed according to the methods provided in the Specification.

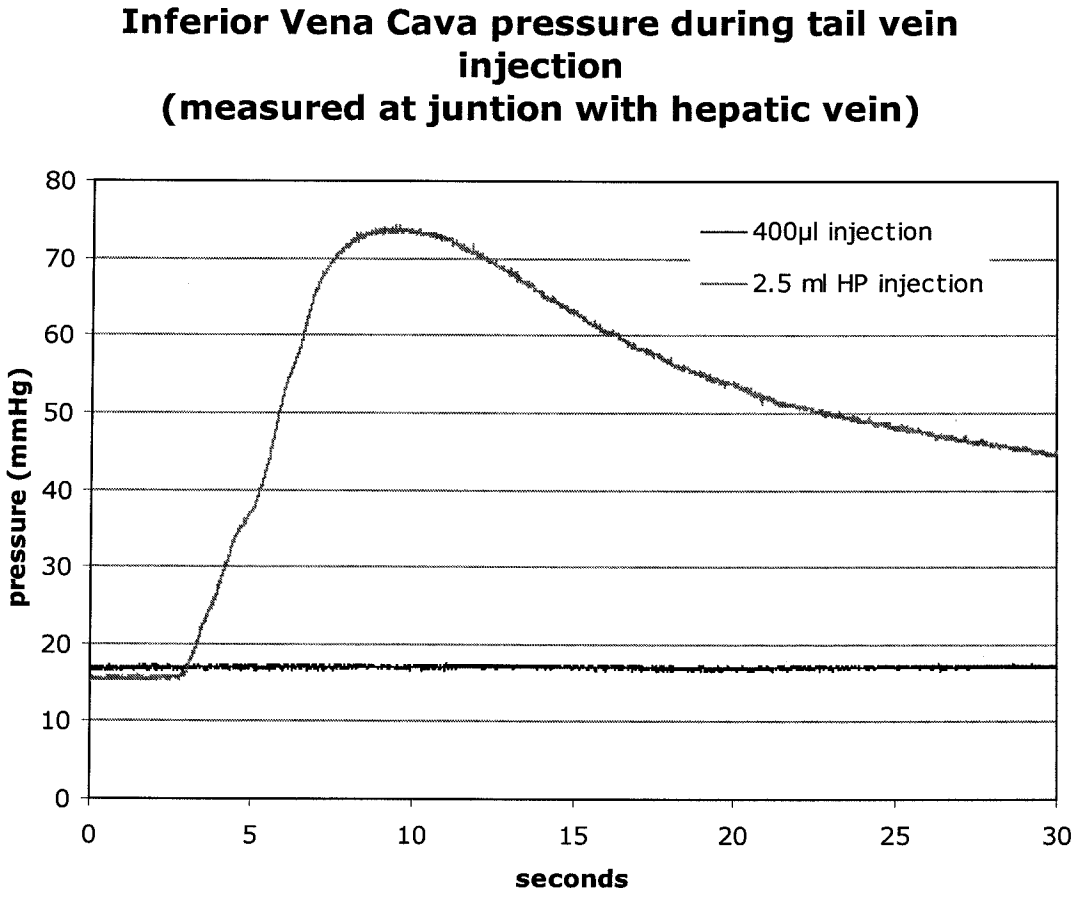
Venous pressure was measured during tail vein injections as follows: Animals were anesthetized with 1-2% isoflurane. A 27 gauge butterfly catheter was placed in the tail vein and secured in place with tape. The abdominal cavity was opened and the intestines were wrapped in moist gauze and exteriorized to expose the renal vein and the Inferior Vena Cava (IVC). The left renal vein and artery (near the kidney) were ligated. A catheter (polyethylene tubing size 10) for measuring intravascular pressure was inserted into the renal vein and advance into the IVC so that the tip was near the hepatic vein. This PE tubing and the IV pressure catheter were connected to a fluid filled calibrated pressure transducer system (EasyGraf, LDS Test and Instruments, Middleton, WI, USA) and the signal output was fed into a data acquisition system (Power lab, ADInstruments, Colorado Springs, Co, USA) so that pressure data could be saved for later analysis. For tail vein injections, injected fluid enters the liver primarily and this site. The intravascular pressure data was collected during the tail vein injection. After the injection, the pressure catheter was removed, the renal vein artery and vein was ligated near the IVC and the left ureter was ligated and the kidney removed. The animals were closed and allowed to recover.

Mouse #1: Typical tail vein injection. A 400 µl solution was injected by hand in about 6-7 seconds.

Mouse #2: Mouse received a typical 400 µl injection followed by a second typical 400 µl injection.

Mouse #3: Mouse received a typical 400 µl followed by a 2.5 ml injection as described in U.S. Application No. 10/007,459.

Injection of 400 µl (more than 3× the volume taught by Zimmer et al.) into the tail vein of a mouse resulted in no measurable increase in intravascular pressure in the Inferior Vena Cava near the junction of the hepatic vein (see graph below). Any pressure in the hepatic vein would have been equal to or less than that measured at this location in the Inferior Vena Cava. Similarly, injection of 400 µl, followed by a second injection of 400 µl, also showed no significant increase in intravascular pressure. Conversely, injection of a larger volume, as described in U.S. Application No. 10/007,459 resulted in a greater than 4.5× increase in intravascular hydrostatic pressure near the liver. All injections were initiated at about the 3 second mark, which corresponds to the start of the rise in pressure measured for the larger volume injection.



I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Vladimir Subbotin 01/28/08 date

Julia Hegge 1/28/08 date

Dr. James Hagstrom 1/29/08 date

Dr. So Wong 1/28/08 date

Dr. Jon A Wolff 2/08/08 date